DOCUMENTED CHROMOSOME NUMBERS 2002: 1. CHROMOSOME NUMBER OF *STENANTHIUM* (LILIALES: MELANTHIACEAE) AND ITS SIGNIFICANCE IN THE TAXONOMY OF TRIBE MELANTHIEAE

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ABSTRACT

Mitotic chromosome counts for *Stenanthium densum* and *S. leimanthoides* (2*n* = 20), reported here for the first time, match the number previously documented for *S. gramineum*, the only other species in this genus as recently recircumscribed based on molecular data. The significance of chromosome numbers in the Melanthiaceae, particularly as generic synapomorphies in tribe Melanthieae, is discussed.

RESUMEN

El recuento de cromosomas mitóticos de *Stenanthium densum* y *S. leimanthoides* (2*n* = 20), reportados por primera vez aquí, concuerdan con el número previamente documentado para *S. gramineum*, la única otra especie de este género recircumscrito recientemente basándose en datos moleculares. Se discute aquí el significado del número de cromosomas en Melanthiaceae, particularmente como sinapomorfías genéricas en la tribu Melanthieae.

The Melanthiaceae sensu the Angiosperm Phylogeny Group (APG 1998) comprise five tribes (11–16 genera; ca. 154–201+ spp.) of predominately woodland and/or alpine perennial herbs occurring mainly in the temperate to Arctic zones of the Northern Hemisphere (with one species of *Schoenocaulon* extending into South America; Zomlefer et al. 2001). Of concern here are two genera of the tribe Melanthieae, *Stenanthium* s.l. and *Zigadenus* s.l. As traditionally and broadly circumscribed, both genera form poorly defined complexes (summary in Zomlefer 1997). *Stenanthium* s.l. has sometimes been divided into two genera: a monotypic *Stenanthium* (*S. gramineum*) and *Stenanthella* (2–4 spp.); *Zigadenus* has a more complex taxonomic history involving several proposed segregates, although contemporary botanists have typically accepted only the monotypic segregate *Amianthium* with the remaining ca. 19 species maintained in *Zigadenus* s.l.

The circumscription and relationships of genera within the tribe Melanthieae were recently evaluated using parsimony analyses of ITS (nuclear ribosomal) and *trnL-F* (plastid) DNA sequence data (Zomlefer et al. 2001). Based on the cladograms generated in this study, *Zigadenus* s.l. is polyphyletic and

Stenanthium s.l. is biphyletic, with *Stenanthium* s.s. and *Stenanthella* embedded within two different "*Zigadenus*" clades. In overview, the species of *Stenanthium* and *Zigadenus*, as traditionally circumscribed, form five strongly supported clades that correlate with distinctive geographical distribution, chromosome number, and certain morphological characters. The *Stenanthium* clade, comprising *Stenanthium gramineum* (*Stenanthium* s.s.), *Zigadenus densus*, and *Z. leimanthoides*, is defined by the morphological synapomorphies of a slender (cylindrical) bulb and one obscure (or lacking) gland per tepal. However, the chromosome number for this group, $2n = 20$, has been documented only for *Stenanthium gramineum* (Miller 1930; Satô 1942; as *S. robustum*, see Fernald 1946). Since chromosome number is a significant feature for genera of tribe Melanthieae, 20 was predicted as the likely mitotic count for the other two species now transferred to the recircumscribed *Stenanthium*, *Stenanthium densum* and *S. leimanthoides* (Zomlefer et al. 2001; Zomlefer & Judd 2002).

MATERIALS AND METHODS

Several plants of *Stenanthium densum* and *S. leimanthoides* were collected during April to June 2001 (see Table 1) and transplanted to pots maintained at the Plant Biology Dept. Greenhouse Facility at the University of Georgia and the Biology Dept. Greenhouse at High Point University. Dividing root tip cells were prepared for examination according to general protocols outlined by Flory and Smith (1980), Jones and Luchsinger (1986), and Smith (1984). Once the plants were well-established, actively growing root tips were harvested at noon and treated with 0.2 % colchicine for 4 hours, rinsed in distilled water, and then fixed in Carnoy's solution (3 ethanol: 1 acetic acid) overnight or longer. Following this fixation, the roots were rinsed in distilled water, hydrolyzed in 1.0 N HCl at 43° C for 25 minutes, macerated with a dissecting spatula on a glass microscope slide, and stained with 1% aceto-orcein. After application of a cover slip, the slide was gently heated with an alcohol lamp, placed between blotters, and subjected to additional pressure. Slides were mounted in euparal for future reference. Well-spread metaphase chromosomes were traced under a Leica DMLB Research Microscope with a camera lucida attachment. Herbarium specimen vouchers (Table 1) are deposited at GA.

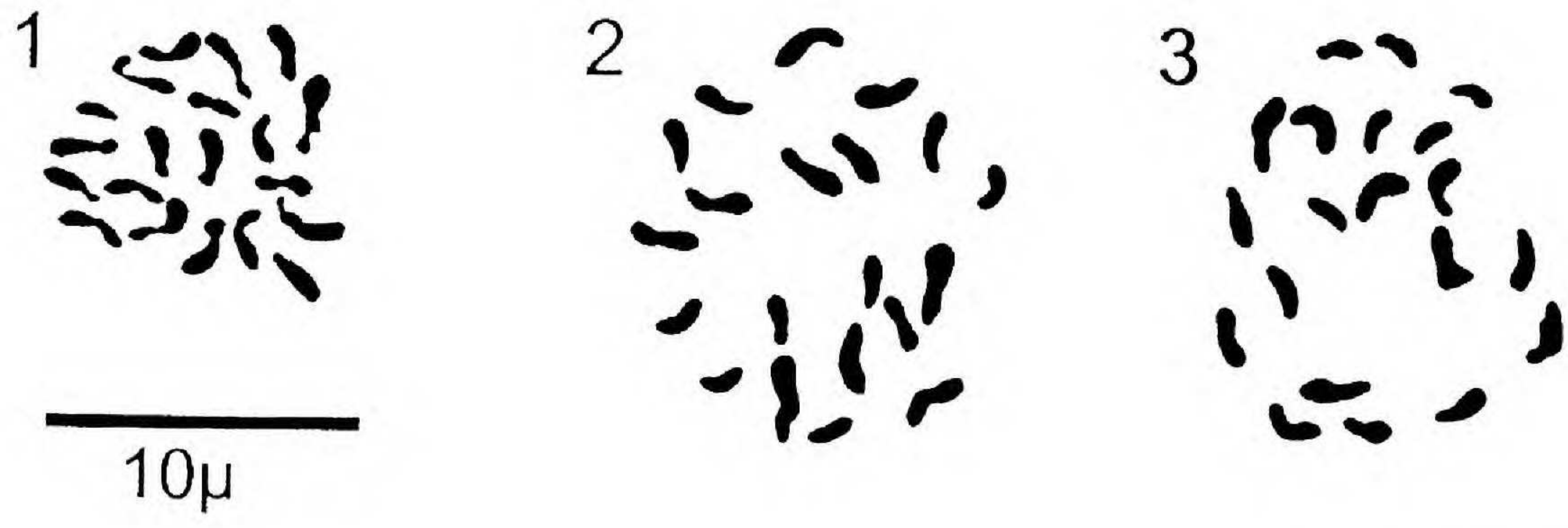
RESULTS AND DISCUSSION

The mitotic chromosome number of $2n = 20$ for *Stenanthium*, as circumscribed by Zomlefer and Judd (2002), is verified for all species (Table 1, Figs. 1–3). Chromosome number is a useful taxonomic character for genera within tribe Melanthieae (see Fig. 4), especially the synapomorphic $2n$ numbers of 20 for *Stenanthium* and 22 for *Toxicoscordion*. A probable base chromosome number of $x = 8$ is often cited for the tribe Melanthieae (Sen 1975; Tamura 1995; Lowry et al.

TABLE 1. Distinguishing features of *Stenanthium densum* and *S. leimanthoides* (from Zomlefer 1997), and voucher specimens for the chromosome numbers reported in this study. Based on reports of morphologically intergrading populations in Mississippi (McDearman 1984), some authors (e.g., Sorrie & Weakley 2001) regard these taxa as conspecific. Despite some overlap in habitat, morphology, and phenology, Small (1903) considered these taxa as two separate monotypic genera (*Tracyanthus* and *Oceanoros*).

Taxon	Distribution, habitat, distinguishing characters, & phenology	Locality & collection number (voucher location)	Mitotic chromosome number (2*n*)
Stenanthium densum (Desr.) Zomlefer & Judd	se VA to e LA Flatwoods, bogs Plants delicate, racemes simple, flowers perfect Apr–May	FLORIDA. Franklin Co.: E side of FL 65, 1.1 mi N of Ft. Gadsden Creek, 4 Apr 2001 *Zomlefer 782* (GA)	20
		FLORIDA. Liberty Co.: W side of FL 379, 0.2 mi N of Forest Service Rd. 180, 4 Apr 2001, *Zomlefer 783* (GA)	20
Stenanthium leimanthoides (A. Gray) Zomlefer & Judd	Wider range: NY to ne TX Pinelands, bogs, montane marshes, swamps Plants strikingly more robust, racemes compound, flowers perfect + staminate (plants andromonoecious) May–June	GEORGIA. Taylor Co.: 4.3 mi N of Butler, 0.25 mi E of GA137, 21 May 2001, *Zomlefer 792* (GA)	20
		GEORGIA. Taylor Co.: 6.1 mi S of Butler, Little Whitewater Creek, W side of GA 137, 18 Jun 2001, *Zomlefer 797* (GA)	20

1987; Zomlefer 1997), and multiples of this number (or perhaps of 4) are prevalent in the group (Fig. 4): *Anticlea* (which includes *Stenanthella*), *Schoenocaulon*, and sister groups *Amianthium* and *Veratrum* (including *Melanthium*). Unfortunately, due to the small chromosome size of members of the tribe (ca. 2.0–4.0 µm in length), the few karyotype studies (e.g., Lee 1985) are not detailed enough to infer a possible mechanism of chromosomal evolution, although the chromosome numbers (Fig. 4) indicate the prevalence of polyploidy and/or aneuploid variation. The functional outgroup for Melanthieae (see Zomlefer et al. 2001), *Z. glaberrimus* (*Zigadenus* s.s.), has an unpublished count of $2n = 52$, tentatively reported by Preece (1956). Probable base numbers for the other immediate outgroup taxa of the tribe Melanthieae (the remaining four tribes of the Melanthiaceae) are five and 15, respectively, for sister taxa Parideae



FIGS. 1–3. Mitotic chromosomes of the three species of *Stenanthium*, all $2n = 20$. 1. *S. gramineum* [modified from Satô (1942), Fig. 58]. 2. *S. densum* (*Zomlefer 782*). 3. *S. leimanthoides* (*Zomlefer 792*).

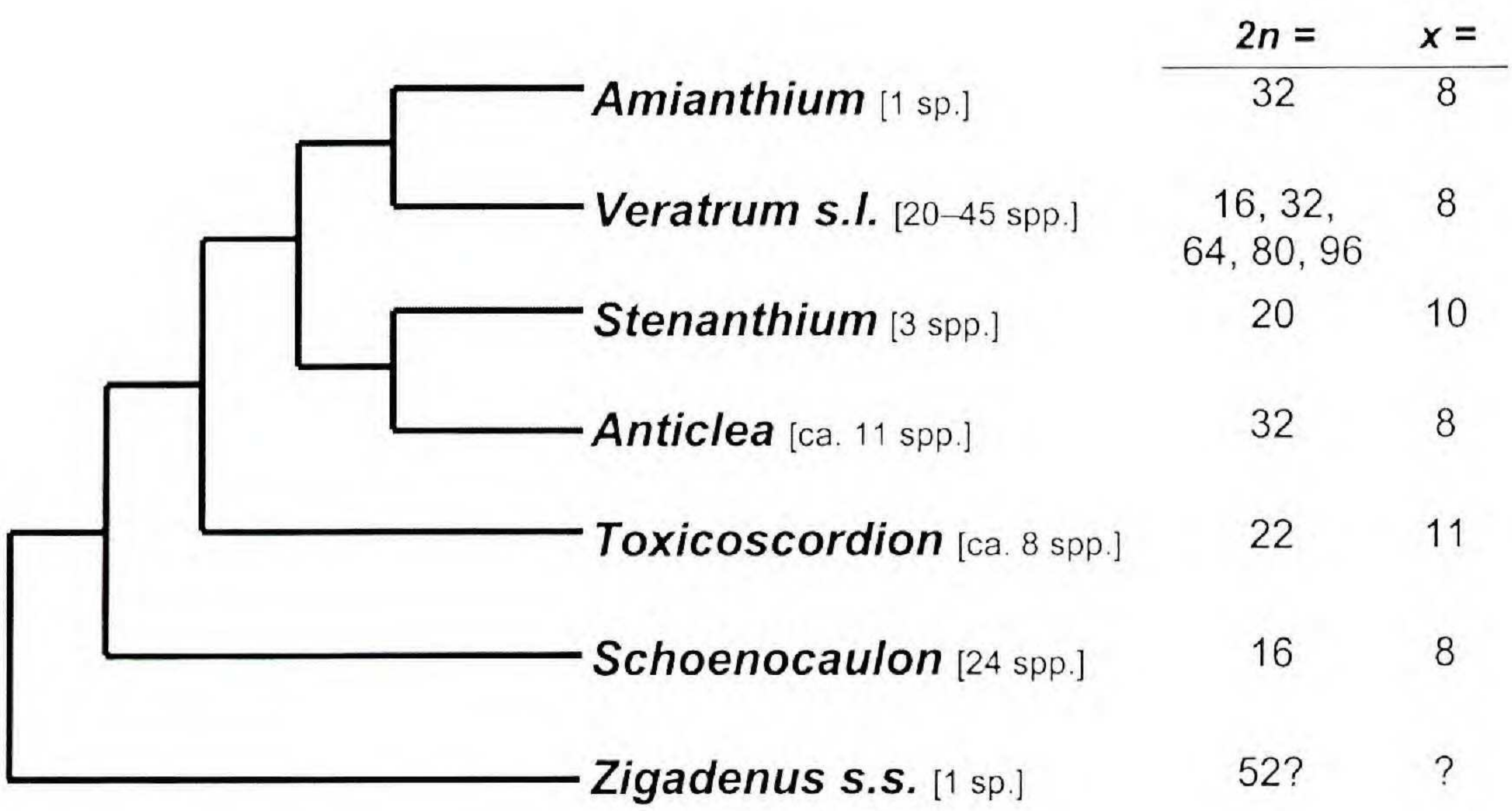


FIG. 4. Phylogenetic relationships and reported chromosome numbers for the genera of tribe Melanthieae. The cladogram, modified from Zomlefer et al. (2001), is based on ITS and *trnL-F* sequence data; chromosome numbers are from references summarized in Zomlefer (1997). As circumscribed here, *Amianthium*, *Stenanthium*, *Anticlea*, *Toxicoscordion*, and *Zigadenus* s.s. all contain species of the polyphyletic *Zigadenus* s.l. (Zomlefer & Judd 2002).

(Trilliaceae) and Xerophylleae, and 17 and possibly six, respectively, for sister taxa Heloniadeae and Chionographideae (Lowry et al. 1987; Zomlefer 1996, 1997).

Confirmation of the chromosome number for *Z. glaberrimus* (a possible polyploid) and the reassessment of the base number for the tribe merit further investigation (Zomlefer in prep.), especially in relation to the probable base numbers of the other tribes. Available data, however, already demonstrate that chromosome number strengthens support for the monophyly of *Toxicoscordion* and *Stenanthium*, as recircumscribed. Chromosome counts for the superficially similar plants in the Melanthieae validate chromosome number as a significant character, useful for elucidating and predicting groupings and relationships. Variation in chromosome number has also proven phylogenetically significant in other monocot clades, such as *Polygonatum*/Polygonateae (Tamura 1993; Tamura et al. 1997a, b).

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